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Occurrence of pathogens in wild rodents caught on Swedish pig and chicken farms

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SUMMARY

A total of 207 wild rodents were caught on nine pig farms, five chicken farms and five non-farm locations in Sweden and surveyed for a selection of bacteria, parasites and viruses. *Lawsonia intracellularia* and pathogenic *Yersinia enterocolitica* were only detected in rodents on pig farms (9% and 8% prevalence, respectively) which indicate that these agents are more likely to be transmitted to rodents from pigs or the environment on infected farms. *Brachyspira hyodysenteriae* (1%), *Brachyspira intermedia* (2%), *Campylobacter jejuni* (4%), *Campylobacter upsaliensis* (2%), leptospires (7%) and encephalomyocarditis virus (9%) were also detected from rodents not in contact with farm animals. *Giardia* and *Cryptosporidium* spp. were common, although no zoonotic types were verified, and *Salmonella enterica* was isolated from 1/11 mice on one farm but not detected by PCR from any of the rodents. *Trichinella* spp. and *Toxoplasma gondii* were not detected.

Key words: Animal pathogens, spread of disease, vectors, veterinary pathogens, zoonoses.

INTRODUCTION

Rodents on farms are unwanted but common inhabitants that may transmit disease-causing agents to domestic animals and indirectly to humans. Rodents can be carriers of diarrhoea-causing agents in pigs, like *Lawsonia* (*La.*) *intracellularis* [1] and *Brachyspira* (*B.*) *hyodysenteriae* [2, 3] and also of various important human pathogens like *Salmonella* and *Trichinella* [4–6]. The aim of this study was to investigate the risk posed by rodents for transmission of pathogens to pig and chicken herds. Rodents were caught on pig farms, chicken farms and non-farm locations (sewage treatment plants, a city mill, a city pond, a supermarket and a veterinary clinic for ruminants) and investigated for the presence of some major bacteria, parasites and viruses by direct isolation or indirect methods like polymerase chain reaction (PCR) and serology.

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The microbes tested for were: the bacteria *La. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli* that are important causes of diarrhoea in pigs; encephalomyocarditis virus, a cardiovirus within the Picornaviridae family that causes sudden death and reproduction problems in pigs and has rodents as a natural reservoir, the foodborne zoonotic agents *Campylobacter* spp., *Salmonella* spp., pathogenic *Yersinia enterocolitica* [7], *Giardia* and *Cryptosporidium*, which cause gastrointestinal infections in humans, and *Trichinella* and *Toxoplasma* (*T.*) *gondii* [8]. Also included in the study were pathogenic leptospires, which are zoonotic bacteria of which some serovars have rodents as a reservoir. Leptospires can cause serious illness in humans and reproduction problems in pigs [9, 10].

Substudies of *Brachyspira* and *Yersinia* have been described previously [11, 12], and the results from these substudies have been included here to provide a complete picture of the pathogens detected in the investigated rodent population.

MATERIALS AND METHODS

Capture of rodents and sampling

The animal procedures were approved by the Swedish Ethical Committee for Scientific Animal Experiments (protocol C247/5). Rodent traps were set at 28 locations (16 pig farms, five chicken farms, seven non-farm locations) as described previously [11]. Both live traps and snap traps were used, baited with peanut butter or chocolate. Animals caught alive were transported in their traps to the laboratory and euthanized by CO_2, while animals caught in snap traps were kept on ice until necropsy. In addition, seven rats killed by a pest animal control agent were included in the study. Sampling and necropsy of the rodents were performed using sterile instruments [11]. Heart, lung, spleen, kidney, liver, brain, lymph nodes and pieces of colon and ileum (0·5 cm from mice, 1·5 cm from rats) were divided in two and frozen at $-80\,^{\circ}C$ or kept in formalin. Samples were collected from the caecum and colon and stored at $+8\,^{\circ}C$ in Amies medium with charcoal (Copan, Italy) or Cary–Blair transport medium [13] until culture. The remainder of the intestines and carcases were used for parasitological examination. Blood was retrieved from the heart and was either stored at $-80\,^{\circ}C$ or stored-dried on filter paper. Bacterial DNA from colon, ileum and kidneys were extracted by Qiagen DNeasy® Blood and Tissue kit (Qiagen, Germany) and viral RNA from cardiac tissue by Qiagen RNeasy® Fibrous Tissue Mini kit (Qiagen).

Bacteria

In brief, *Campylobacter* spp. were analysed from swab samples from the colon by direct culture on mCCDA (Oxoid, UK) according to ISO method 10272:1:2006. From each suspected positive sample, at least one typical *Campylobacter* colony was subcultured and confirmed as thermophilic *Campylobacter* spp. [14]. A multiplex PCR was used for identification and differentiation of the thermophilic species *Campylobacter* (*C.*) *jejuni* and *C. coli* [15], while for isolates of uncertain identity, three additional PCRs were applied [16–18]. *Salmonella enterica* was analysed by real-time PCR [19] on DNA prepared directly from colon samples, without pre-enrichment. In addition, culture by ISO method 6579:2002 was performed on the intestinal contents of a subpopulation of mice ($n=11$) originating from a laying-hen farm experiencing an outbreak of *S.* Typhimurium. Analysis for *Campylobacter* and *Salmonella* was performed at the Department of Bacteriology, National Veterinary Institute, Uppsala, Sweden. Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* were identified by culture of colon samples and TaqMan PCR of colon DNA, and genetic fingerprinting by pulsed-field gel electrophoresis of isolates from rodents and pigs was performed [11]. *Leptospira* spp. in DNA extracted from kidneys was analysed by conventional PCR, encoding the *hap1* gene present in pathogenic strains [20]. All positive PCR products were sequenced with ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA). For the detection of *La. intracellularis*, DNA extracted from the colon and ileum was analysed by a species-specific PCR [21]. A mimic was used as an internal control to detect the presence of inhibition [22]. Three positive PCR products were sequenced with an ABI Prism 3100 Genetic Analyzer. The isolation, phenotyping and characterization of *B. hyodysenteriae*, *B. pilosicoli* and *B. intermedia* have been described in a previous paper [12].

Parasites

Trichinella spp. were analysed by the magnetic stirrer digestion method (EC 2075/2005) in whole carcasses from mice and in ~ 25 g of muscle tissue from rats and one vole. IgG antibodies against *T. gondii* were

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studied by direct agglutination test with the Toxo-Screen DA kit (bioMérieux, France). Dry-stored blood was dissolved in PBS before analyses. All samples were run in duplicate. *Giardia* cysts and *Cryptosporidium* oocysts in intestinal contents were detected simultaneously with a fluorescein-labelled direct immunofluorescence kit, Aqua-Glo^{TM}G/C (Waterborne^{TM} Inc., USA). Molecular characterization of *Giardia* isolates by sequencing the genes for β-giardin, *gdh*, *tpi* and ssu-rRNA has been described in a previous paper [23]. In addition, amplification of the ssu-rRNA gene [24] with subsequent sequencing was performed on four *Cryptosporidium* isolates. Parasitological analyses were performed at the Department of Virology, Immunobiology and Parasitology, National Veterinary Institute, Uppsala, Sweden, and characterization of *Giardia* and *Cryptosporidium* spp. at the Department of Diagnostics and Vaccinology, Swedish Institute for Communicable Disease Control, Stockholm, Sweden.

Virus

RNA extracted from cardiac tissue was analysed for encephalomyocarditis virus (EMCV) by RT–PCR with the Qiagen® OneStep RT–PCR kit (Qiagen, Germany) [25]. Sequencing was performed on five amplicons using ABI PRISM BigDye Terminator v. 3.1. and was run on a Genetic Analyzer 3100 (Applied Biosystems, USA).

RESULTS AND DISCUSSION

In this study, the presence of a number of human and pig pathogens was investigated in 207 rodents caught on nine pig farms, five chicken farms and, for comparison purposes, five non-farm locations. At nine of the 28 visited locations, of which seven were pig farms, no rodents were trapped despite repeated efforts. Judging from faecal droppings and burrows, rodents were common inhabitants on all farms visited, but in practice traps could not always be placed at the most strategic points. In addition, active pest control was applied on all farms visited, usually directly by a pest control company, which regularly replenished bait stations with rat poison. Finally, the conditions differed regarding, e.g. free access to the farm for trapping, tidiness on the farm and presence of cats, all of which probably affected the trapping outcome.

Of the 207 rodents, 32 were caught in live traps, 168 in snap traps, and seven rats were killed by a pest animal control agent. The rodent species caught were house mice, *Mus musculus* ($n=129$; 50 males, 72 females, seven unspecified sex), brown rats, *Rattus norvegicus* ($n=58$; 35 males, 22 females, one unspecified sex), yellow-necked mice, *Apodemus flavicollis* ($n=19$; nine males, 10 females) and one water vole, *Arvicola terrestris* (female), the latter was caught in an urban location.

Pathogens identified

The number of samples included in the different analyses varied for technical and practical reasons. For the analysis of EMCV and *Leptospira* spp. by PCR, a limited number of samples (125 and 127, respectively) were selected from pig farms where 10 or more rodents had been caught, with non-pig locations as reference. The results are presented in relation to rodent species and type of location in Table 1.

Regarding the important causes of diarrhoea in pigs, *La. intracellularis* was detected by PCR in 5% of all rodents (seven house mice, two brown rats), all caught on pig farms (prevalence in rodents on pig farms 9%). Comparison of the sequences of three amplicons with uploaded sequences in GenBank showed 100% identity with *La. intracellularis* PHE/MN1-00 (GenBank accession no.AM180252), which confirms the high similarity in isolates from various animal species as shown by Cooper *et al.* [26]. All rodents trapped on chicken farms and other premises tested negative for *La. intracellularis* in the present study, which suggests that rodents are infected by pigs. Faecal shedding of *La. intracellularis* can continue for up to 3 weeks in rats and mice after challenge infection [27]; rodents could therefore pose a risk of re-introduction after an eradication programme.

As we have reported previously, *B. hyodysenteriae*, the aetiological agent of swine dysentery was recovered from brown rats, and *B. pilosicoli*, causing colonic spirochaetosis was recovered from rats and mice [12]. The results confirm previous findings that rats and mice are carriers of *Brachyspira* spp. [2, 3] and thus wild rodents in particular should be considered a risk for the introduction and spread of swine dysentery.

Regarding foodborne zoonoses, *Campylobacter* spp. were isolated from 33 (16%) of the rodents tested and eight were identified as *C. jejuni*, while 25 were designated as unspecified thermophilic *Campylobacter*

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Table 1. *Number and percentage of positive samples related to rodent species and type of location*

Pathogen	Brown rat	House mouse	Yellow-necked mouse	Water vole	Pig farms	Chicken farms	Other locations	Total
Lawsonia intracellularis	2/55 (4%)	7/115 (6%)	0/19 (0%)	0/1 (0%)	9/105 (9%)	0/53 (0%)	0/24 (0%)	9/182 (5%)
Brachyspira hyodysenteriae	1/58 (2%)	0/129 (0%)	0/19 (0%)	0/1 (0%)	0/116 (0%)	0/59 (0%)	1/32 (3%)	1/207 (1%)
Brachyspira pilosicoli	2/58 (3%)	1/129 (1%)	0/19 (0%)	0/1 (0%)	3/116 (3%)	0/59 (0%)	0/32 (0%)	3/207 (1%)
Brachyspira intermedia	3/58 (5%)	1/129 (1%)	0/19 (0%)	0/1 (0%)	1/116 (1%)	0/59 (0%)	3/32 (9%)	4/207 (2%)
Campylobacter jejuni	2/58 (3%)	2/125 (2%)	5/18 (28%)	0/1 (0%)	3/114 (3%)	5/58 (9%)	1/31 (3%)	9/203 (4%)
Campylobacter coli	5/58 (9%)	15/125 (12%)	0/1 (0%)	0/1 (0%)	19/114 (17%)	1/58 (2%)	0/31 (0%)	20/203 (9·8%)
Campylobacter upsaliensis	0/58 (0%)	2/125 (2%)	2/18 (11%)	0/1 (0%)	2/114 (2%)	0/58 (0%)	2/31 (6%)	4/203 (2%)
Salmonella enterica	0/56 (0%)	0/115 (0%)	0/13 (0%)	0/1 (0%)	0/107 (0%)	0/54 (0%)	0/24 (0%)	0/185 (0%)
Yersinia enterocolitica	7/56 (13%)	2/120 (2%)	0/13 (0%)	0/1 (0%)	9/111 (8%)	0/55 (0%)	0/25 (0%)	9/191 (5%)
Yersinia pseudotuberculosis	0/55 (0%)	1/120 (1%)	0/13 (0%)	0/1 (0%)	1/111 (1%)	0/55 (0%)	0/25 (0%)	1/191 (0·5%)
Cryptosporidium spp.	6/50 (12%)	10/98 (10%)	1/7 (14%)	0/1 (0%)	16/106 (15%)	0/34 (0%)	1/16 (6%)	17/156 (11%)
Giardia spp.	14/50 (28%)	6/98 (6%)	1/7 (14%)	0/1 (0%)	12/106 (11%)	1/34 (3%)	8/16 (50%)	21/156 (13%)
Trichinella spp.	0/49 (0%)	0/104 (0%)	0/7 (0%)	0/1 (0%)	0/113 (0%)	0/33 (0%)	0/15 (0%)	0/161 (0%)
Toxoplasma gondii	0/49 (0%)	0/86 (0%)	0/12 (0%)	0/1 (0%)	0/89 (0%)	0/35 (0%)	0/24 (0%)	0/148 (0%)
Leptospira spp.	1/51 (2%)	6/68 (9%)	1/5 (20%)	1/1 (100%)	7/105 (7%)	0/5 (0%)	2/17 (12%)	9/127 (7%)
Encephalomyocarditis virus	2/40 (5%)	9/78 (12%)	0/7 (0%)	0/0 (0%)	10/97 (10%)	1/17 (6%)	0/11 (0%)	11/125 (9%)

by the hippurate test [28]. PCR verified the identity of the *C. jejuni* isolates and, of the previously unspecified isolates, 20 were identified as *C. coli*, one as *C. jejuni* and four as *C. upsaliensis*. Interestingly, several isolates of *C. jejuni* and *C. upsaliensis* were obtained from yellow-necked mice, a wild rodent species that in contrast to the house mouse and the brown rat has its natural habitat in woodlands. However, it is not known whether this species acts as a reservoir for these *Campylobacter* spp. *C. jejuni* and *C. coli* were isolated from rodents captured on pig farms and chicken farms, but *C. jejuni* was more common on chicken farms and *C. coli* on pig farms, which might suggest that rodents pick up the bacteria from the farm animals. However, when Meerburg and colleagues used amplified fragment-length polymorphism to analyse *C. coli* from pig manure and rodents on organic farms, no identical genotypes were identified from the two species [6]. In the present study, genotyping of the isolates obtained was not performed, and it is therefore unknown whether the rodents carried pathogenic strains. Still, the findings in this study imply that rodents should be considered a risk factor for introduction of *Campylobacter* spp. into chicken and pig houses. One isolate of *S. enterica* serovar Typhimurium was obtained by cultivation of faecal samples from 11 mice captured on a laying-hen farm that had recently experienced an outbreak of salmonellosis. However, when using PCR directly applied on colonic DNA without pre-enrichment, *S. enterica* could not be detected in this sample or in any of the other samples. It should be emphasized that the PCR method used was developed for analysis of presumptive colonies [19], and potential *Salmonella* bacteria might have been present in numbers too low to be detected. Ideally, cultivation using pre-enrichment media should be used. Furthermore, inhibitory substances could have been present in the samples. Other studies have reported high prevalence of *Salmonella* in rodents, especially on contaminated poultry farms [29, 30], but negative results or low prevalence on farms with unknown *Salmonella* status [6, 31]. Sweden has a favourable situation regarding *Salmonella*, with only a few positive herds detected each year [32] and the importance of wild fauna as a source of infection is marginal [32, 33]. Thus, the lack of detection in the present study seems logical and may reflect the true situation, but further studies are required to fully estimate the risk of rodents as a source of *Salmonella*.

As described previously [11], 9% of the rodents caught on pig farms were positive for pathogenic

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Y. enterocolitica by PCR, and genetic fingerprinting by pulsed-field gel electrophoresis showed that rodent isolates of *Y. enterocolitica* 4/O:3 were highly similar to pig isolates. *Y. pseudotuberculosis*, of unknown prevalence in Swedish pigs, was detected in one house mouse caught on an outdoor pig farm.

Unspecified *Cryptosporidium* spp. were detected in 11% of the rodents analysed. All rodent species tested positive and the majority of infected individuals were caught on pig farms. Analysis of four isolates by sequencing of the ssu-rRNA gene showed identical sequences to *Cryptosporidium* (*Cr.*) *muris*, *Cryptosporidium* sp. rat genotype (FJ205699), an environmental *Cryptosporidium* isolate (AY737585) and an isolate with 99% similarity to a strain isolated from drinking water (HM015877). The sequences obtained in this study have been deposited in GenBank (accession nos. JN172968–JN172971). No zoonotic types were demonstrated, but only a minority (24%) of the positive samples were characterized. Thus, no certain conclusions can be drawn regarding zoonotic relevance, but there may well be an interchange of parasites between pigs and rodents, and between rodents and the environment. *Giardia* cysts were detected in all rodent species (13%). Analyses by sequencing of the genes for *β-giardin*, *gdh*, *tpi* and ssu-RNA, as described previously [23], conferred cysts from three samples to the species *Giardia muris* and eight samples from brown rats to *G. intestinalis* assemblage G. Cysts from one sample from a yellow-necked mouse were *G. microtis*-like (differing by four single nucleotide polymorphisms at the ssu-rRNA locus). Cysts from nine additional rodents (five house mice, four brown rats) were detected but were not further characterized. The prevalence of *Giardia* spp. in rodents was high in the present study, but there was no evidence of transmission of zoonotic variants.

Neither *Trichinella* spp. nor *T. gondii* were detected in this study. The negative result for *Trichinella* spp. was not surprising, considering the absence of trichinosis in Swedish pig production. The latest case in domestic pigs was recorded in 1994, while sporadic cases are still found in wild boars. In Sweden during 2009, *Trichinella* spp. was found in wolverines, wolves and lynxes [32]. It can be speculated that the rodent populations studied here have their habitat mostly indoors on farms or are urban, and therefore do not come in contact with the sylvatic cycle. However, it would be interesting to examine a larger number of yellow-necked mice, which move between their wild habitat and pig houses. The negative result for *T. gondii* serology was a little surprising, since several other studies based on serology have shown high prevalence in rodents [34], although there are also contradictory reports [35]. In Sweden, antibodies against *T. gondii* have been found in wildlife such as moose, roe deer [36], free-ranging Eurasian lynx [37], pet cats, dogs, horses [38] and pigs [39], so we expected to find antibodies in rodents too. One possible explanation is that the prevalence of *Toxoplasma* has decreased in the Swedish pig population since 2002, when Lundén *et al.* reported a seroprevalence of 3·3% in fattening pigs and 17·3% in adult swine [39]. It is also possible that serology is not the optimal method for an epidemiological survey for *T. gondii*. Other studies have shown that the prevalence in rodents might be underestimated when relying on serology compared to isolation [34] or PCR [40], moreover, some studies have shown rats with viable *T. gondii* to be seronegative [34]. In a recent study by Meerburg *et al.*, an infection rate of 9% in house mice on farms was indicated based on real-time PCR analysis [41]; however, in that study the locations were organic farms with an outdoor system whereas in the present study the majority of farms were conventional.

Nine (7%) samples were detected by PCR as pathogenic leptospires. Positive samples originated from six house mice and one yellow-necked mouse caught at four different pig farms, and one rat and one water vole caught at urban locations. Sequencing of the PCR products confirmed the existence of several genetic variants with 98–100% similarity to deposited sequences of *Leptospira* (*L.*) *borgpetersenii*, *L. weili* or *L. interrogans* serovar Copenhageni in GenBank. The brown rat is generally considered the maintenance host for serovar Icterohaemorrhagiae, and small rodents such as the house mouse as maintenance hosts for serovar Sejroe [42]. Leptospirosis is an important zoonosis in many countries, but in Sweden domestic cases of leptospirosis in humans are extremely rare (data from Swedish Institute for Communicable Disease Control, Smittskyddsinstitutet) [43]. In pigs, *Leptospira* serovar Bratislava has been detected to be related to reproductive problems in a few cases, but seems to be of minor importance [44–46]. In the present study, the prevalence in rodents was lower than reported previously. In Croatia, Cvetnić *et al.* found 23% of house mice positive by serology [47] and Krojgaard and colleagues detected *Leptospira* spp. by PCR in 53% of sewer rats in Copenhagen [48]. However, detection method and the type of location differ between the studies, so direct comparison is difficult.

In RT–PCR analyses, 11 (9%) samples tested positive for EMCV. Both house mice and rats tested positive and sequencing of PCR products showed 97–100% similarity to EMCV sequences in GenBank. The findings support the hypothesis that rodents are natural reservoirs of EMCV [49]. To our knowledge, clinical outbreaks have not been reported in Sweden, but the present results show that EMCV should be considered as a possible differential diagnosis in cases of sudden death in growing pigs or reproductive problems of unclear aetiology, especially when rodents are abundant.

In summary, this study found that brown rats and house mice in Sweden carry *C. jejuni*, *C. coli*, *C. upsaliensis*, *Y. enterocolitica*, *La. intracellularis*, *B. hyodysenteriae*, *B. pilosicoli*, *B. intermedia*, *Leptospira* spp. and EMCV. Rodents, whether they are reservoirs or carriers of these pathogens, can spread pathogenic bacteria and viruses between different locations, directly to farm animals and, for the zoonotic species, indirectly to humans. The negative results for *Trichinella* spp. and *T. gondii* and the very low prevalence of *S. enterica* can be related to low prevalence of these pathogens in Swedish farm animals. In conclusion, the risk for transmission of pathogens by rodents should be seriously considered when deciding on hygiene barriers on farms, and thus rodent pest control should be an important measure in order to maintain good biosecurity.

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DECLARATION OF INTEREST

None.

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